SYNTHESIS OF FRAGMENTS OF THE HISTONE FRACTIONS H1 AND H2b CONTAINING THE Ser-106 AND Ser-14 RESIDUES

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One of the elements of the regulation of gene activity is the process of modifying histones, which is effected by special enzymes and, in particular, by histone kinase. The latter specifically phosphorylates the Ser-37 residue in histone fraction H1 and the Ser-14 and Ser-36 residues in histone fraction H2b [1, 2]. Furthermore, a hypothesis of the phosphorylation of Ser-106 in histone H1 has been put forward [3].

In order to study the specificity of the action of histone kinase, we have synthesized two fragments from histone fractions H1 and H2b. The first fragment (103-108) includes the Ser-106 residue, and the second fragment (11-16) the Ser-14 residue:

H-Ala-Ser-Gly-Ser-Phe-Lys-OMe (A)

H-Lys-Lys-Gly-Ser-Lys-Lys-OMe (B)

In the synthesis of these compounds we used serine with an unprotected hydroxy group (Schemes 1 and 2). Although cases are known in the literature of the use in peptide synthesis of serine with an unprotected OH group [4, 5], we nevertheless approached the realization of the schemes mentioned above with great circumspection, since not infrequently on the use of dicyclohexylcarbodiimide (DCHCD) side reactions connected with the open hydroxy group of a serine residue are observed. In actual fact, when Z-Ala-Ser-OH was condensed with H-Gly-Ser-Phe-OCH_3 hydrobromide by Scheme 1, the chromatogram showed the presence of by-products in addition to the main product Z-Ala-Ser-Gly-Ser-Phe-OCH_3. By paper chromatography we preparatively isolated two substances with identical amino-acid compositions. It was assumed that the impurity may be a depsipeptide formed through the free OH group of a serine residue. In this case, it could be expected that the depsipeptide bond would readily be hydrolyzed in 1 N NaOH. In actual fact, under these conditions the "depsipeptide" by-product disappeared and we obtained the chromatographically pure peptide Z-Ala-Ser-Gly-Ser-Phe-OH. It is interesting to note that in the production of compound (B) we observed no such anomalies connected with an unprotected OH group.

EXPERIMENTAL

The initial amino acids were the L forms. The homogeneity of the peptides synthesized was determined by TLC on plates with a fixed layer of silica gel (25 × 75 mm, 250 mesh) and on "Silufol UV-254" plates (Czechoslovakia) in the following systems: 1) butan-1-ol–water–acetic acid (100:30:10); 2) butan-2-ol–3% aqueous NH_3 (100:44); 3) methanol–chloroform (13:60); and 4) butan-1-ol–acetic acid–pyridine–water (15:3:10:12). The chromatograms were revealed with a 0.5% solution of ninhydrin and with iodine vapor. The electrophoretic investigation of compounds with free amino groups was performed on Whatman 2 paper at pH 2.7 in 0.2 N acetic acid with a voltage gradient of 38 V/cm for 45 min. The peptides were hydrolyzed in 6 N hydrochloric acid at 105°C for 24 h. The circular dichroism spectra were taken on a "Jobin Yvon" dichrograph.

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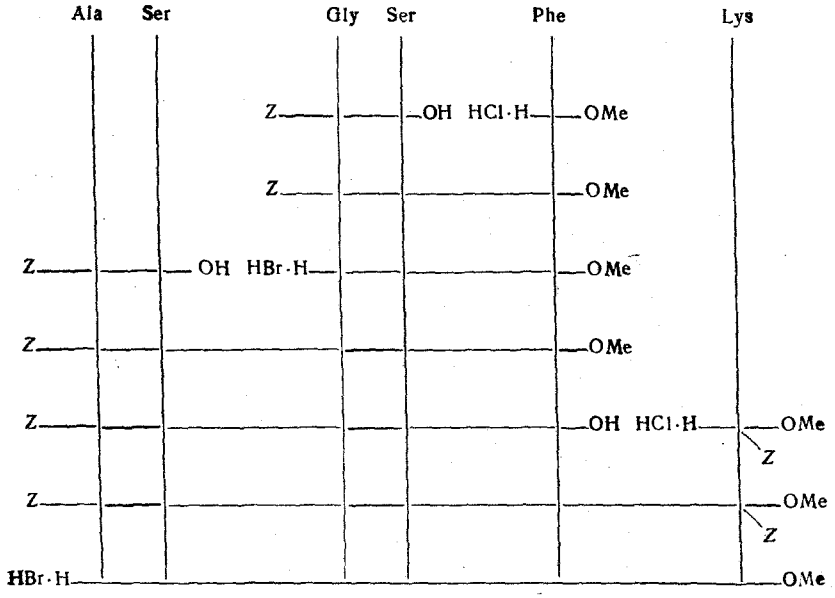
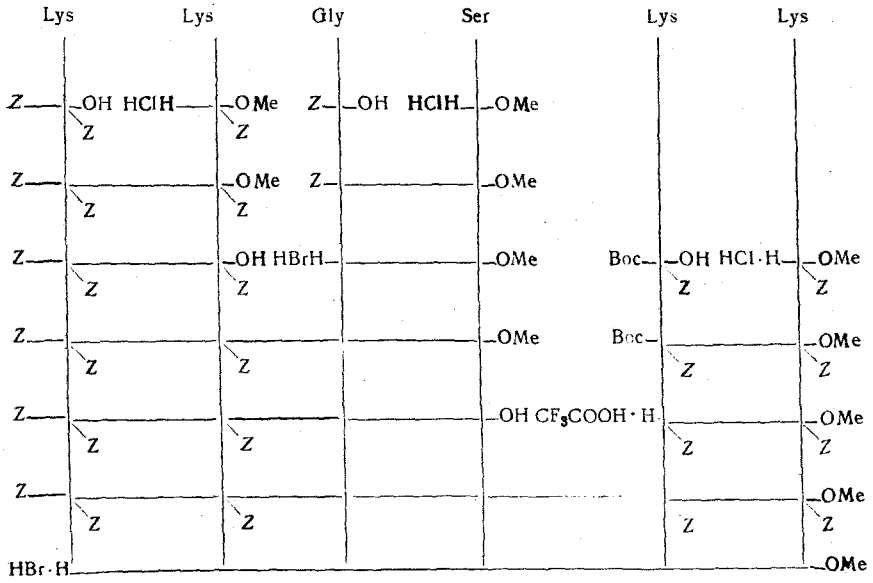


Scheme 1



Scheme 2

Methyl Ester of Benzyloxycarbonylserylphenylalanine (I). A solution of 6 g of benzyloxycarbonylglycylserine [6] in 15 ml of tetrahydrofuran (THF) cooled to −10°C was treated with 4.2 g of DCHCD, and, with stirring, 4.4 g of the hydrochloride of the methyl ester of phenylalanine [7] in 5 ml of THF containing 2.8 ml of triethylamine (TEA) was added. The mixture was stirred at 0°C for 1 h and was left overnight at 20°C. The dicyclohexylurea was filtered off, the filtrate was evaporated, the residue was dissolved in ethyl acetate, and the solution was washed with 1 N hydrochloric acid (3× 15 ml), with 0.5 N sodium bicarbonate (3× 15 ml), and with water, and was dried over sodium sulfate. The solvent was evaporated in vacuum, and the residue was dissolved in the minimum volume of methanol and was precipitated with ether. The yield of amorphous product (I) was 6.3 g (68%), $[\alpha]^{22}-28°$ (c 1; chloroform); R_f 0.61, 0.80, 0.78 in systems 1, 3, and 4, respectively.

Hydrobromide of the Methyl Ester of Glycylserylphenylalanine (II). Hydrogen bromide was passed into a solution of 5 g of the methyl ester of benzyloxycarbonylglycylserylphenylalanine in 20 ml of absolute nitromethane for 30 min. Then absolute ether was added and the precipitate that deposited was washed with ether by decantation (3 × 20 ml), dissolved in the minimum volume of absolute chloroform, and reprecipitated with absolute ether. This gave 3.2 g (73%) of the amorphous product (II), hygroscopic in the air, R_f 0.25 in system 3.

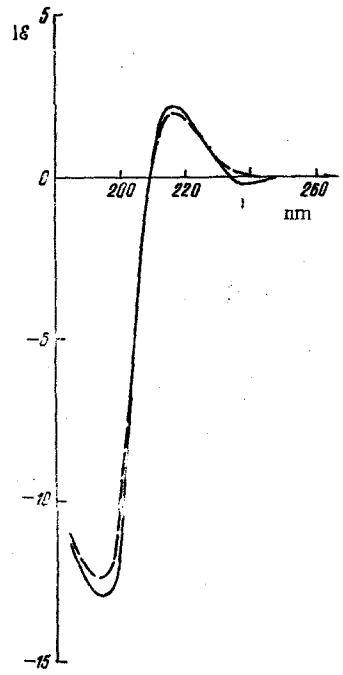


Fig. 1. CD curves: 1) HBr · H-Ala-Ser-Gly-Ser-Phe-Lys-OMe, 2) HBr · H-Lys_2-Gly-Ser-Lys_2-OMe in phosphate buffer, 0.001 M, pH 7.0, 25°C.

Methyl Ester of Benzyloxycarbonylalanylserylglycylserylphenylalanine (III). At −10°C, 1.1 g of DCHCD and, with stirring, 2.1 g of the hydrobromide of the methyl ester of glycylserylphenylalanine in a mixture of 5 ml of dimethylformamide (DMFA) and 2 ml of methylene chloride were added to a solution of 1.6 g of benzyloxycarbonylalanylserine [6] in 10 ml of DMFA. The product was worked up as for (I). After recrystallization from chloroform–ether, 1.6 g (51%) of (III) was obtained with mp 125-128°C, R_f 0.55 in system 2, $[\alpha]^{22}$ −22.4° (c 0.98; chloroform).

Benzyloxycarbonylalanylserylglycylserylphenylalanine (IV). Over 20 min, with stirring, 25 ml of 0.1 N caustic soda was added in portions to a solution of 1 g of the methyl ester of benzyloxycarbonylalanylserylglycylserylphenylalanine in 25 ml of ethyl acetate. The aqueous layer was then separated off, and the ethyl acetate layer was washed with water (3 × 5 ml). The combined aqueous extracts were cooled to −0°C and acidified with 4 N hydrochloric acid to pH 2. The oil that deposited was extracted in the cold with ethyl acetate (3 × 20 ml), and the extract was dried over sodium sulfate and evaporated in vacuum. The residue was dissolved in the minimum volume of chloroform and was reprecipitated with ether. The yield of amorphous product (IV) was 0.6 g (57%), $[\alpha]^{22}$ −12.4° (c 0.94, chloroform), R_f 0.4 and 0.58 in systems 3 and 4, respectively.

Methyl Ester of Benzyloxycarbonylalanylserylglycylserylphenylalanyl(N^{ε}-benzyloxycarbobyl)lysine (V). This was obtained similarly to (I) from 0.44 g of benzyloxycarbonylalanylserylglycylserylphenylalanine in 5 ml of THF, 0.15 g of DCHCD, and 0.23 g of the hydrochloride of the methyl ester of N^{ε}-benzyloxycarbonylysine [8] in 3 ml of THF containing 0.1 ml of TEA.

After the elimination of the solvent, the oily residue was triturated with ether until it solidified and it was then filtered off, dried, and crystallized from a mixture of chloroform and ether. The yield of crystalline product (V) was 0.3 g (48%), mp 68-70°C; R_f 0.83, 0.82, and 0.79 in systems 2, 3, and 4, respectively, $[\alpha]^{22}$ −25.8° (c 0.93; chloroform).

Hydrobromide of the Methyl Ester of Glycylserine (VI). A current of hydrogen bromide was passed through a solution of 3 g of the methyl ester of benzyloxycarbonylglycylserine in 10 ml of nitromethane for 35 min. Then absolute ether was added, the precipitate that deposited was washed by decantation with ether (3 × 20 ml), and it was reprecipitated from chloroform with ether. This gave 2.3 g (92%) of the crystalline product (VI) with mp 146-149°C, R_f 0.51 and 0.45 in systems 1 and 2, respectively.

Methyl Ester of Benzyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysylglycylserine (VII). This was obtained similarly to (I) starting from 2.8 g of benzyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysyl-

(N^{ε}-benzyloxycarbonyl)lysine [9] in 15 ml of THF, 0.85 g of DCHCD, and 1.07 g of the hydrobromide of the methyl ester of glycylserine in 5 ml of THF containing 0.58 ml of TEA.

After recrystallization from a mixture of ethyl acetate and ether, 2.9 g (84%) of product (VII) was obtained with mp 118-120°C; Rf 0.88 and 0.67 in systems 3 and 4, respectively; $[\alpha]^{25}$ −11.7° (c 0.89; DMFA).

Benzyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysylglycylserine (VIII). The synthesis was performed similarly to that of (IV), starting from 1.3 g of the methyl ester of benzyloxycarbonyl-(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysylglycylserine in 20 ml of ethyl acetate and 20 ml of 0.1 N caustic soda. The reaction time was 20 min. After elimination of the solvent, the oily residue was triturated with petroleum ether, and the resulting solid was filtered off, dried, and recrystallized from a mixture of ethyl acetate and petroleum ether. This gave 1.1 g (78.5%) of a crystalline product (VIII) with mp 152-154°C, Rf 0.85 and 0.83 in systems 3 and 4, respectively $[\alpha]^{22.5}$ −14° (c 0.86, DMFA).

Methyl Ester of Benzyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysylglycylseryl-(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysine (IX). To a solution of 1 g of benzyloxycarbonyl-N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysylglycylserine in 5 ml of THF at −10°C were added 0.25 g of DCHCD and 0.82 g of the trifluoroacetate of the methyl ester of (N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysine [10] in 3 ml of THF containing 0.17 ml of TEA. The mixture was stirred at 0°C for 1 h, left at 20°C for 24 h, and worked up similarly to (I). After recrystallization from a mixture of ethanol and ether, 1.3 g (0.79%) of the crystalline product (IX) was obtained with mp 143-145°C; Rf 0.86 and 0.79 in systems 3 and 4, respectively, $[\alpha]^{22.5}$ −21.2° (c 0.98, DMFA).

Hydrobromide of the Methyl Ester of Alanylserylglycylserylphenylalanyllysine (X). A current of hydrogen bromide was passed through a solution of 100 mg of the methyl ester of benzyloxycarbonylalanylserylglycylseryl-phenylalanyl(N^{ε}-benzyloxycarbonyl)lysine in 2 ml of abs. CF_3COOH for 30 min. Then 5 ml of benzene was added and the mixture was evaporated in vacuum at 30°C. The residue was dissolved in 1 ml of H_2O and the solution was passed through a column of silica gel. Elution was carried out with the buffer solution butan-1-ol–acetic acid–pyridine–water (15 : 3 : 10 : 12). The rate of elution was 12 ml/h. The fraction collected was freeze-dried, giving 15 mg (37%) of an amorphous product with Rf 0.46 in system 4.

Hydrobromide of the Methyl Ester of Lysyllysylglycylseryllysyllysine (XI). Compound (XI) was obtained similarly to (X), starting from 100 mg of the methyl ester of benzyloxycarbonyl(N^{ε}-benzyloxycarbonyl)lysyl-glycylseryl(N^{ε}-benzyloxycarbonyl)lysyl(N^{ε}-benzyloxycarbonyl)lysine in 2 ml of absolute TFA. The product obtained was passed through a column of silica gel and was eluted with a buffer solution of butan-1-ol–acetic acid–pyridine–water (15 : 3 : 10 : 12). The rate of elution was 15 ml/h. This gave 22 mg (43%) of an amorphous product with Rf 0.41 in system 4.

Methyl Ester of Alanylserylglycylserylphenylalanine (XII). Compound (XII) was obtained similarly to (X), starting from 100 mg of the methyl ester of benzyloxycarbonylalanylserylglycylserylphenylalanine in 2 ml of TFA. The time of passage of hydrogen bromide was 25 min. After the addition of absolute ether, an amorphous product deposited which was washed by decantation with ether (3 × 50 ml), and the residue was reprecipitated from ethanol with ether. The amorphous final residue was dissolved in methanol and was passed through a column of IRA resin in the OH^- form. The solvent was evaporated off and the residue was reprecipitated from ethanol with ether, giving 55 mg (53%) of an amorphous residue with Rf 0.33 and 0.78 in systems 2 and 4, respectively.

SUMMARY

1. Peptide fragments forming analogs of sequences 103-108 of histone H1 and 11-16 of histone H2b have been synthesized.

2. The formation of a depsipeptide was observed in the synthesis of peptide (A) but not in that of peptide (B).

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THE PRECIPITATION OF ISOLATED COTTON-PLANT PROTEIN

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The search for additional sources of protein to make up its deficiency in feeding regions is an urgent problem. A promising source of protein is formed by cotton seeds and cottonseed meal. The quality and quantity of the protein obtained from the meal depends on the conditions of its extraction, precipitation, and drying. As is well known, the most acceptable methods of separating protein from an extract on the industrial scale is its precipitation at the isoelectric point.

The isoelectric points of protein substances are not constant magnitudes and depend largely on the conditions of extraction, i.e., on the amount and nature of the accompanying substances present in the extract. We have studied the precipitation of protein from an extract obtained by treating cottonseed meal with a 5% solution of NH_4Cl at pH 5.7-6.0 [1].

In all the experiments we used meal from the Kokand Oils and Fats Combine. The protein was extracted at room temperature, and clarified extract was used. To determine the optimum medium for the precipitation of the protein we performed a series of experiments on a large laboratory apparatus. In each experiment, 20 liters of protein extract was placed in a precipitating vessel and, under the same conditions, the protein was precipitated by varying the pH of the extract with added 10% hydrochloric acid. The protein precipitate was separated off on a centrifuge and was washed with water, and was then defatted.

The results of the experiments are given below

pH of the precipitate	1	2	2,5	3	3,5	4	4,5	5
Yield of protein on the weight of the initial meal, %	13,5	14,3	14,45	14,55	14,57	14,14	8,1	—

The maximum precipitation of the protein is observed at pH 3-3.5 (90% of the dissolved protein substances is precipitated), which does not correspond with the isoelectric point of cottonseed protein obtained by extraction in an alkaline medium, which is 4.2 [2, 3]. Furthermore, the protein obtained by extraction in an alkaline medium sharply changes its solubility with a fall in the pH, and at pH 1.5 and below the precipitated protein dissolves completely, which was not observed in our experiment.

The cause of the sharp change in the solubility of the protein at low pH values of the medium is the presence of phytin in the extracted protein obtained on extraction in a weak alkaline medium [4]. Experiments to determine the dependence of the protein-precipitating process on the nature of the acid have shown that the yield of protein does not depend on this factor. Hydrochloric acid is used in practice in the food industry and is more economical for the precipitation of protein.

We used for precipitation a 10% solution of hydrochloric acid, as in the method for precipitating soya protein [5].

We also studied the influence of the temperature and the time of coagulation on the precipitation of protein, for which, in each experiment, 20 liters of extract was placed in the precipitating vessel and the protein was precipitated under similar conditions but with variation in the temperature of the extract:

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